Theriogenology 131 (2019) 169–176

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Heat stress impact on the expression patterns of different reproduction related genes in Malabari goats

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ARTICLE INFO

Article history:
Received 23 March 2018
Received in revised form
22 March 2019
Accepted 30 March 2019
Available online 2 April 2019

Keywords:
Climate change
ESTRα
Heat stress
PGF2α
COX-2
Reproductive trait

ABSTRACT

A study was conducted to evaluate the effect of heat stress on the expression pattern of reproduction related genes in Malabari breed of goat. The study was conducted during 45 days using twelve 10 months to one year old Malabari goats. The goats were randomly allocated into two groups: MC (n = 6; Malabari control) and MHS (n = 6; Malabari heat stress). Goats were stall-fed with a diet composed of 60% roughage and 40% concentrate. All animals had access to *ad-libitum* feed and water and they were fed and watered individually. The MC goats were placed in the shaded pens while MHS goats were exposed to heat stress in outside environment between 10.00 h and 16.00 h. At the end of study period, all 12 animals were slaughtered and their uterus tissues were collected for gene expression and histopathological studies. The temperature humidity index (THI) inside shed (74.9) proved that the animals were not stressed while in the outside environment (86.5) the animals were extremely distressed. Heat stress significantly ($P < 0.05$) influenced the expression patterns of follicle stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR), estrogen receptor α (*ESTRα*), prostaglandin F2 α (*PGF2α*) and cyclooxygenase-2 (COX-2). However, prostaglandin E2 (PGE2) did not differed between the groups. Further, a strong positive correlation ($P < 0.01$) was established for THI with both *FSHR gene* expression. A negative correlation was also established between THI and *ESTRα* ($P < 0.01$), *PGF2α* ($P < 0.01$), *PGE2* ($P < 0.05$) *genes*. However, heat stress did not influence the expression patterns of *LHR*; *COX-2 genes*. The histopathological section of uterine epithelial cells showed degenerative changes ($P < 0.05$) with less differentiation in MHS group as compared to MC group. The results from the study clearly indicated that heat stress was able to alter the reproductive activity related gene expressions at uterine level and this could be an indication of reduced reproductive efficiency in Malabari goats.

1. Introduction

The livestock sector plays a crucial role for the livelihood security of the farming community. The livestock acts as the source of revenue for 1.3 billion poor people [1]. Currently, climate change was considered as the most threatening factor affecting the welfare of the livestock. Among all the climatic variables, heat stress can be considered as the most detrimental factor to the livestock population [2]. Although the animals possess the capabilities to adapt to the changing climate, they do so by compromising their productive functions especially the reproduction so as to deviate energy resources for maintaining the life sustaining activities [3].

Among all the livestock species, small ruminants play a vital role in securing the livelihood of rural community as they offer meat, milk, offal and wool [4]. Goats possess the higher resilience capacity than cattle and sheep due to their higher thermo-tolerance, drought tolerance, ability to survive on limited pastures and highly disease resistance capability [5,6]. Further, goats possess higher feed conversion efficiency and have the better ability than other ruminant species to efficiently convert the feed resources into either meat or milk [7,8]. Moreover, indigenous goats were found to be more adapted than the crossbred or pure bred animals [9]. Therefore, it is very vital to study the adaptive capacity of local goat breeds in an effort to identify the most suitable breed for a specific

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https://doi.org/10.1016/j.theriogenology.2019.03.036
0093-691X/

location.

The impact of heat stress on reproduction has been widely established cutting across species particularly in dairy cattle [10], buffaloes [11], sheep [12] and goat [13]. From the study undertaken by Gwazdauskas [14] clearly emphasis that the heat stress imparts a detrimental effect on livestock reducing the conception rate by 20%–27%. Similarly, the elevated temperature affects the secretion of gonadotropin which leads to inadequate production of estrogen and progesterone [15]. Several studies have established the adverse impacts of heat stress on the endometrial functions and secretary activities in various livestock species [16]. Thus, the imbalance in secretions and low progesterone level [17] could lead to early expression of luteolytic mechanism and further fails in implantation [18]. Similarly, heat stress condition induces significant changes in the production of adrenocorticotropic hormone (ACTH) and prostaglandin F2α (*PGF2*α). Further, heat stress also was established to negatively influence the follicular dynamics leading to compromised reproductive performance [16].

Although, several studies established the impact of heat stress on reproduction based on changes in the phenotypic traits, researches on the implications of the same based on genotypic traits are very scarce. In addition, the underlying molecular mechanisms by which heat stress induced changes in the genetic traits pertaining to animal reproduction are not elucidated. Hence it is high time that such research efforts are oriented towards establishing the basic molecular mechanism of heat stress induced changes in animal reproduction.

Therefore, this study attempts to reveal the hidden intricacies associated with the heat stress induced changes in the reproductive traits of indigenous Malabari goat breed. The study was conducted with the primary objective of establishing the effect of heat stress on expression patters of different traits that controls Malabari goat reproduction. The study may yield suitable genetic markers governing reproduction during heat stress exposure. Identification of such markers through genetic selection and incorporating those genes in thermo-sensitive breeds may increase the possibilities of higher productivity in heat stressed animals.

2. Materials and methods

2.1. Study site

The experiment was conducted in the experimental livestock unit of the ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India located on latitude 77°36′25.3″E, longitude 12°57′04.3″N and altitude of 920 m above mean sea level. The mean annual maximum and minimum ambient temperature of this regime ranges between 15 and 36 °C respectively. The mean annual relative humidity ranges from 20 to 85%. The average annual precipitation in this region varies between 200 and 970 mm with erratic distribution. The average annual minimum and maximum temperature ranges between 15 and 22 °C and 27-34 °C respectively. The study was conducted during the month of April to May. The maximum-minimum temperatures, relative humidity, dry and wet bulb temperature, pen surface temperature and temperature-humidity index (THI) during the study period (45 days) are listed in Fig. 1. The calculations of THI was based on the formula Temperature-Humidity Index (THI) = 0.72 (Tdb + Twb) + 40.6 [19] and as per this methodology the THI between 72 and less are comfortable; values between 75 and 78 are stressful and values above 78 are considered extreme distress.

2.2. Animals

A total of 12 ten months to one year old Malabari female goats weighing between 12 and 19 kg were used. Malabari goat breed is a meat purpose animal originated in the humid tropical region of southern India. The animals were brought from different locality and acclimatized to the current experimental location for a period of 45 days. These animals were maintained in well ventilated sheds following standard farm management procedures.

2.3. Experimental design

The study was conducted for a period of 45 days. Twelve animals were used in this study. The animals were randomly allocated into two groups of six animals each, MC (n = 6; Malabari control), and MHS (n = 6; Malabari heat stress). The animals were stall fed with a diet consisting of 60% roughage (Hybrid Napier) and 40% concentrate (Maize 36 kg, wheat bran 37 kg, soybean meal 25 kg, mineral mixture 1.5 kg, common salt 0.5 kg/100 kg). The chemical composition of diet offered to the experimental animals are described in Table 1. The MC animals were maintained in the shed in thermoneutral condition while MHS animals were exposed outside to summer heat stress between 10:00 h to 16:00 h during the experimental period. The MC animals were fed and watered inside the shed while MHS animals were fed and watered while they are exposed to summer heat stress in the outside environment. All cardinal weather parameters were recorded twice daily both inside and outside the shed throughout the study period. The animals were slaughtered at the end of the study and their uterus samples were collected for gene expression study. The study was conducted after obtaining approval from the institute ethical committee for subjecting the goats to summer heat stress.

2.4. Expression of reproduction related genes in uterus

2.4.1. Principle

Samples were lysed and homogenized in lysis buffer, containing guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogenous RNases. The lysate is then mixed with ethanol which disrupts the screening of charges thereby facilitating the binding of RNA with silica. Pure RNA was then eluted under low ionic strength conditions with nuclease-free water [20].

2.4.2. Sample collection and storage

Immediately after the slaughter the uterus samples (n = 6 in each group) were collected. The samples were cut into small pieces, washed in phosphate buffered saline and immersed in RNA shield (Zymo Research, USA) and snap chilled in liquid nitrogen within 5 min of slaughter. Then samples were shifted and stored at −80 °C till further use.

2.4.3. Sample preparation for RNA isolation

After thawing, the tissues were removed from the RNA shield (Zymo Research, USA) and immediately processed for RNA isolation. The total RNA was isolated from tissues using the GeneJET RNA purification kit (Thermo Scientific, Lithuania) and the procedure was done as per manufacturer's protocol with slight modifications as follows: About 30 mg of tissues were pulverized and homogenized with a Cole-Parmer LabGEN DTH homogenizer in lysis buffer after treating with liquid nitrogen (−196 °C). After homogenization, 300 μL of lysis buffer supplemented with β-mercaptoethanol (10 μL/ml) was added and the content was transferred to 1.5 ml microcentrifuge tube. The lysate was vortexed for 10 s. To the lysate, 10 μL of proteinase K in 590 μL of Tris Ethylenediaminetetraacetic Acid buffer was added, then vortexed and incubated at 15–25 °C for 20 min. Then, the contents were centrifuged for 8 min at 12,000 g and the supernatant was transferred into a new RNase-free micro centrifuge tube. Thereafter, 450 μL of ethanol was added and mixed

Table 1
Ingredients and chemical composition of concentrate mixture and hybrid Napier hay fed to goats.

Attribute	Concentrate mixture (kg/100 kg)	Napier hay (*Pennisetum purpureum*)
Ingredients		
Maize	36	–
Wheat bran	37	–
Soybean meal	25	–
Mineral mixture	1.5	–
Salt	0.5	–
Chemical composition (%)		
Dry matter	92.9 ± 0.079	94.0 ± 0.289
Organic matter	95.9 ± 0.190	95.4 ± 0.298
Crude protein	19.6 ± 0.176	6.21 ± 0.098
Ether extract	1.82 ± 0.183	1.49 ± 0.026
Total ash	4.10 ± 0.190	4.64 ± 0.298
Fibre fractions (%)		
Neutral detergent fibre	40.4 ± 1.400	82.9 ± 0.881
Acid detergent fibre	11.1 ± 0.239	64.6 ± 1.950
Acid detergent lignin	2.14 ± 0.029	12.3 ± 0.651
Nutritive value		
Total digestible nutrients %[a]	72.2	55.0
Digestible energy (kJ/kg)[a]	13.3	10.1
Metabolizable energy (kJ/kg)[a]	10.9	8.28

[a] Calculated values.

well by pipette. Then 700 μL of lysate was transferred to a spin column with a 2 ml collection tube and centrifuged for 1 min at 12,000 g. After discarding the flow through, 700 μL of wash buffer 1 was added and centrifuged for 1 min at 12,000 g followed by two time washing with 600 and 250 μL of wash buffer 2 followed by centrifugation at 12,000 g for 1 and 2 min, respectively. About 25 μL of warm nuclease-free water was added to the membrane, and centrifuged at 10,000 g for 1 min to elute RNA. The purified RNA samples were stored at −80 °C until cDNA synthesis.

2.4.4. DNase treatment

Total RNA isolated from different tissues was treated with DNase (TURBO DNA-free, Ambion, USA) in order to eliminate the genomic DNA contamination in total RNA. During and after DNase treatment, 1 μL of RNase inhibitor (20 U/μL, Invitrogen, USA) was added. After DNase treatment quality and quantity of the isolated RNA was analyzed using NanoDrop spectrophotometer. Integrity of the total RNA was checked using denaturing agarose gel (1%) electrophoresis and visualization under UV light. Two intact bands of 28 s and 18 s indicated good quality and intactness of RNA.

2.4.5. cDNA synthesis

The total RNA of was reverse transcribed into cDNA using Maxima first strand cDNA synthesis kit for Real Time quantitative polymerase chain reaction (RT-qPCR) (Thermo Scientific, Lithuania). The 1.5 μg of total RNA was used for cDNA synthesis using random hexamer primers. The procedure was performed as per manufacturer's protocol with modifications are as follows: 4 μL of 5x Reaction Mix, 2 μL Maxima Enzyme Mix, 1.5 μg of Template RNA was used for uterus sample and nuclease-free water were added into a sterile microfuge RNAase-free tube to make up the volume to 20 μL. Then the contents were mixed gently and centrifuged and subjected to reverse transcribing PCR (10 min at 25 °C, followed by 20 min at 50 °C and the reaction was terminated by heating at 85 °C for 5 min). The product of the first strand cDNA synthesis was diluted to a final concentration of 25 ng/1 μL with nuclease-free water and 2 μL of diluted cDNA was used for each reaction in RT-qPCR.

2.4.6. Primer design and synthesis

Gene specific primers were designed using online NCBI primer design software (Primer3, http://bioinfo.ut.ee/primer3/) and specificity was checked using Primer3 and BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The preferences were given to the primers binding to the exon-exon junction. The efficiency of the primers was checked using pcrEFFICIENY - an open source PCR efficiency prediction [21]. The primers were titrated with different concentrations (0.8, 0.4, 0.2, 0.1, and 0.05 μM) for selecting optimum concentration to be used for RT-qPCR experiments. Table 2 describes the primers used for gene expression studies. Primers used for amplifying the target regions of various genes are given in Table 2.

2.4.7. Quantification by RT-qPCR analysis

The relative expression of selected genes was studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA). The 20 μL reaction was carried out in triplicates using 50 ng of template RNA, 10 μl containing Maxima SYBR Green/rox qPCR Master mix (2x) containing 2.5 mM MgCl2 and 0.1–0.2 μM primer concentrations. The RT-qPCR reaction conditions were: enzyme activation at 95 °C for 10 min and amplification cycle (40 cycles; initial denaturation at 95 °C for 15 s, annealing for COX-2 at 56 °C and at 60 °C for 30sec, and extension at 72 °C for 30 s) using Applied Biosystems StepOnePlus Real-Time PCR Systems. The melt curve analysis was performed to check the non-specific amplification. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH), Hypoxanthine phosphoribosyl transferase 1 (HPRT1), and SDHA- Succinate dehydrogenase complex flavoprotein subunit A, gene was used as reference genes and the relative expression was analyzed using the formula, 2ΔΔCT [22].

2.5. Statistical analysis

The changes in relative expression of different genes in relation to the reference gene were analyzed using SPSS (16.0) software using one-way analysis of variance (ANOVA). The significance level was set at $P < 0.05$. Further, the correlation coefficient between the THI and all genotypic traits were established by Pearson's correlation coefficient test using SPSS (version 18.0) software. The R^2 values were used to establish the correlation association between THI and various genetic traits with two levels of statistical significance set at $P < 0.01$ and $P < 0.05$.

Table 2
Primers used for FSHR, LHR, ESTRα, COX-2, PGF2α and PGE2 gene expression. GAPDH is used as reference gene to normalize the gene expression of candidate genes.

Gene ID	Primers	Primer sequence (5′- 3′)	Primer Length (bp)	Product Size (bp)	Ta (°C)	Accession No	Primer concentration (nM)
FSHR	F	ATGCGGTCGAACTGAGGTTT	20	146	60	NM_001285636.1	100
	R	GGGCAGGTTGGAGAACACAT	20				
LHR	F	TTCCACTAAACTGCAGGCCC	20	147	60	NM_001314279.1	100
	R	CAGTGGCTGGGGTAAGTCAG	20				
ESTRα	F	ATACGAAAAGACCGCCGAGG	20	109	60	GQ 358923.1	100
	R	GGTTGGCAGCTCTCATGTCT	20				
COX2	F	GCCTAGCACTTTCGGTGGAG	20	134	56	JN 743538.1	200
	R	CCGTTTTGGTGAGGTGCGTA	20				
PGF2α	F	AGTTTGGAACAGATGCCCCC	20	123	60	XM 005678194.3	100
	R	GCTGGCCACTCAAGTCATCT	20				
PGE2	F	TGCTCCTTGCCTTTCACGAT	20	138	60	NM_001314255.1	200
	R	AGGAGGTCTCAGGATGGCAA	19				
GAPDH	F	GGTGATGCTGGTGCTGAGTA	20	265	60	AF030943	100
	R	TCATAAGTCCCTCCACGATG	20				
HPRT1	F	GCCCCAGCGTGGTGATTAG	19	145	60	XM_018044253.1	Newly synthesized
	R	ACATCTCGAGCCAGTCGTTC	20				
SDHA	F	GCTAAAGTTTCAGATGCGATTTCTG	20	128	60	XM_018065656.1	Newly synthesized
	R	CGT GTT GAA ACC TGC TTC GG	20				

Note: Ta– Annealing Temperature, bp-base pairs, F– forward, R-reverse, the candidate genes are: FSHR- Follicle stimulation hormone receptor; LHR- Luteinizing hormone receptor; ESTRα - Estrogen receptor α; COX-2- Cyclooxygenase 2; PGF2α- Prostaglandin F2 α; PGE2- Prostaglandin E2; GAPDH - Glyceraldehyde 3-phosphate dehydrogenase HPRT1 - hypoxanthine phosphoribosyltransferase 1, SDHA- Succinate dehydrogenase complex flavoprotein subunit A.

3. Results

3.1. Temperature-humidity-index

The THI values both inside and outside the shed in the morning are 69.9 ± 0.16 and 73.5 ± 0.14 respectively while in the afternoon the values were 74.9 ± 0.56 and 86.5 ± 0.39 respectively. The THI inside shed proved that the animals were not stressed while in the outside environment they were extremely distressed. This difference in THI between inside and outside the shed were highly significant ($P < 0.01$).

3.2. Relative follicle stimulating hormone receptor (FSHR) mRNA expression pattern

Fig. 1(A) describes the heat stress impact on the expression patterns of *FSHR* in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus *FSHR* in control and heat stress groups are 1.04 and 0.08 respectively. The heat stress significantly ($P < 0.05$) influenced the expression pattern of *FSHR* in Malabari goat with lower value established being in MHS group. Further, a positive correlation ($P < 0.05$) was established between THI and *FSHR gene* expression pattern (Table 3).

3.3. Relative luteinizing hormone receptor (LHR) mRNA expression pattern

Fig. 1(B) depicts the heat stress impact on the expression patterns of *LHR* in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus *LHR* in control and heat stress groups are 1.0 and 0.29 respectively. The heat stress significantly ($P < 0.05$) influenced the expression pattern of *LHR* in Malabari goat with lower value established being in MHS group. Further, no significant correlation was established between THI and *LHR* gene expression pattern (Table 3).

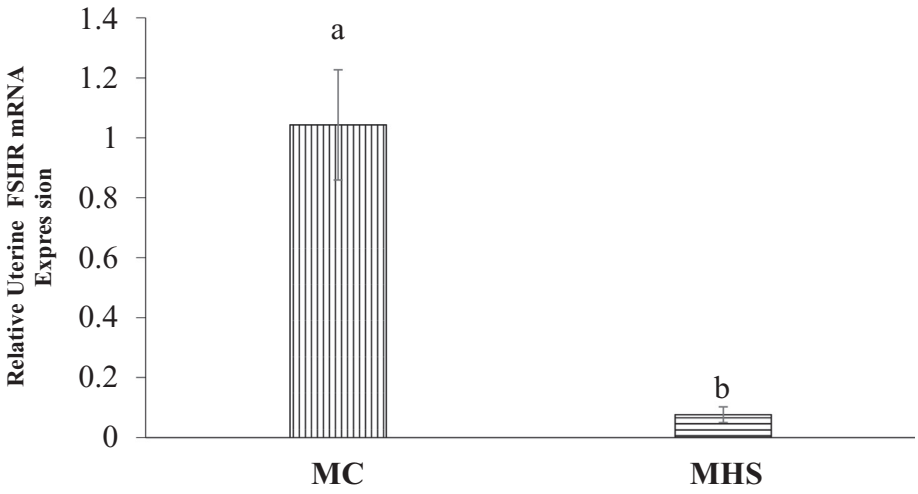

Fig. 1A. . Relative quantitative expression patterns of uterus FSHR mRNA in both control and heat stressed Malabari goats.
Fig.1(A) depicts the impact of heat stress on the FSHR mRNA expression patterns between control and heat stress group of Malabari goats. MC-Malabari control; MHS-Malabari heat stress and FSHR-Follicle stimulating hormone receptor. The values bearing different superscripts differ significantly at $P < 0.05$.

Table 3
Correlation association between THI and reproduction related gene expression.

	THI	FSHR	LHR	ESTRα	COX2	PGF2α	PGE2
THI	1						
FSHR	0.83*	1					
LHR	−0.49	0.08	1				
ESTRα	−0.99**	−0.76	0.59	1			
COX2	0.73	0.73	−0.22	−0.78	1		
PGF2α	−0.98**	−0.70	0.63	0.97**	−0.61	1	
PGE2	−0.87*	−0.45	0.86	0.92**	−0.56	0.94**	1

THI- Temperature humidity index; FSHR- Follicle stimulation hormone receptor; LHR- Luteinizing hormone receptor; ESTRα - Estrogen receptor α; COX-2- Cyclo-oxygenase 2; PGF2α- Prostaglandin F2 α; PGE2- Prostaglandin E2.
**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$.

3.4. Relative estrogen receptor α (ESTRα) mRNA expression pattern

Fig. 1(C) describes the heat stress impact on the expression patterns of ESTRα in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus ESTRα in control and heat stress groups are 1.04 and 5.28 respectively. Further, it was evident that the ESTRα expression pattern was significantly ($P < 0.05$) up regulated in heat stress group as compared to the control group animals. Further, a strong negative correlation ($P < 0.01$) was established between THI and ESTRα gene expression pattern (Table 3).

3.5. Relative cyclooxygenase-2 (COX-2) mRNA expression pattern

Fig. 1(D) illustrates the heat stress impact on the expression

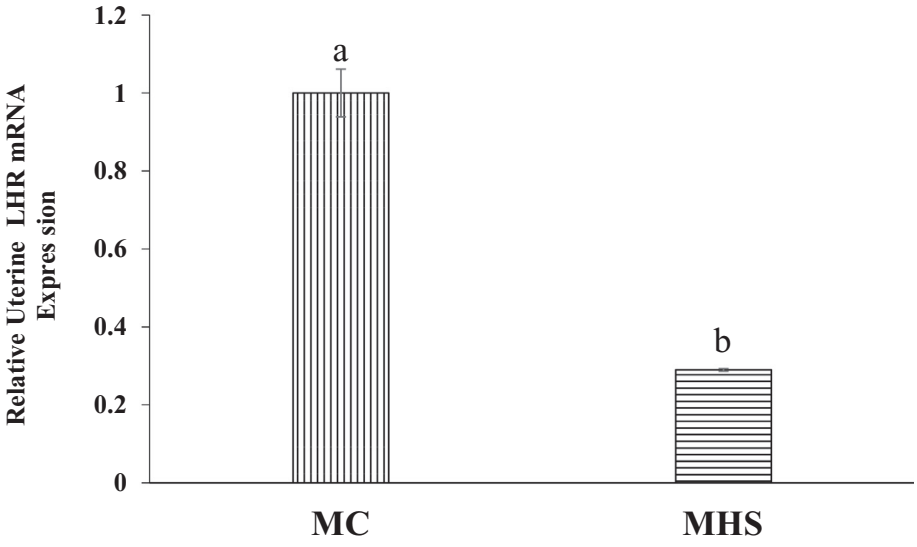

Fig. 1B. . Relative quantitative expression patterns of uterus LHR mRNA in both control and heat stressed Malabari goats.
Fig.1(B) depicts the impact of heat stress on the LHR mRNA expression patterns between control and heat stress group of Malabari goats. MC-Malabari control; MHS-Malabari heat stress and LHR-Luteinizing hormone receptor. The values bearing different superscripts differ significantly at $P < 0.05$.

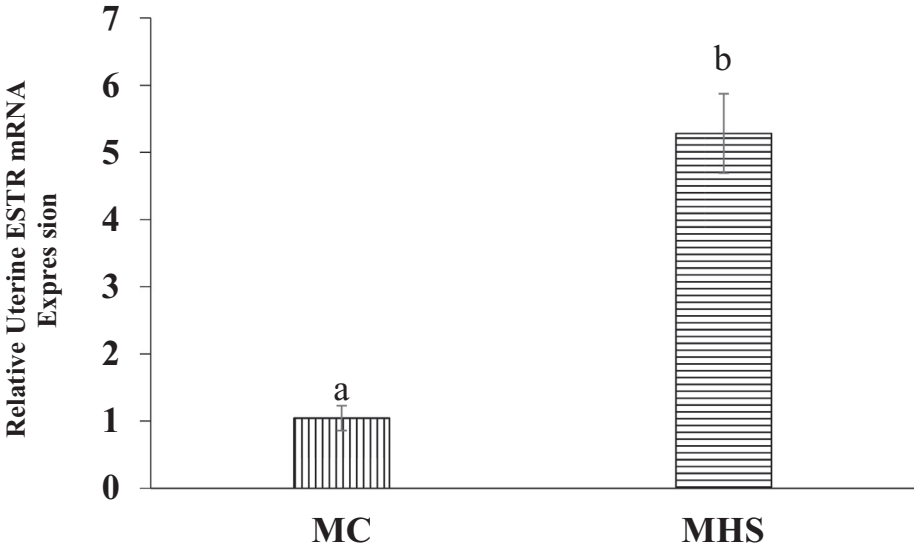

Fig. 1C. . Relative quantitative expression patterns of uterus ESTRα mRNA in both control and heat stressed Malabari goats.
Fig.1(C) depicts the impact of heat stress on the ESTRα mRNA expression patterns between control and heat stress group of Malabari goats. MC-Malabari control; MHS-Malabari heat stress and ESTRα-Estrogen receptor α. The values bearing different superscripts differ significantly at $P < 0.05$.

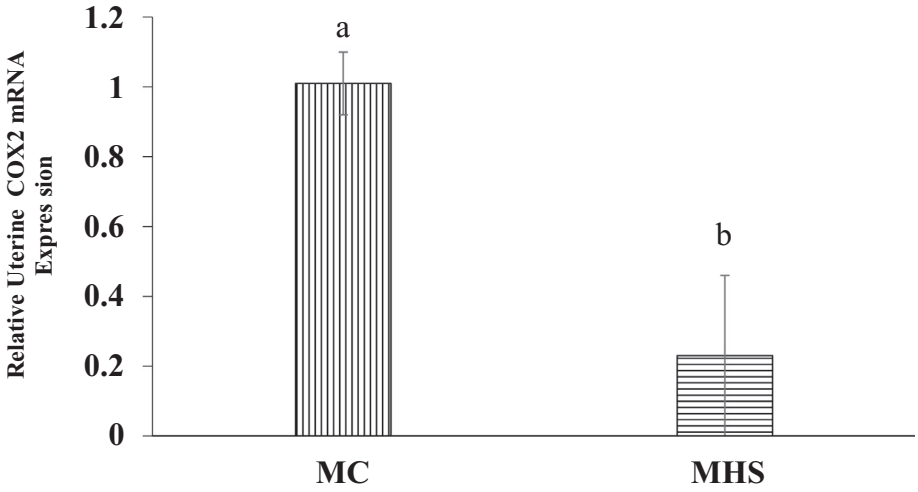

Fig. 1D. . Relative quantitative expression patterns of uterus COX2 mRNA in both control and heat stressed Malabari goats.
Fig.1(D) represents the impact of heat stress on the COX-2 mRNA expression patterns between control and heat stress group of Malabari goats. MC-Malabari control; MHS-Malabari heat stress and COX-2- Cyclooxygenase-2. The values bearing different superscripts differ significantly at $P < 0.05$.

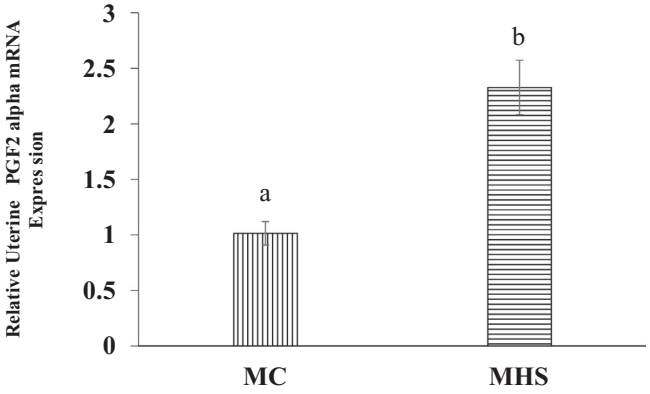

Fig. 1E. . Relative quantitative expression patterns of uterus PGF2α mRNA in both control and heat stressed Malabari goats.
Fig.1(E) depicts the impact of heat stress on the PGF2α mRNA expression patterns between control and heat stress group of Malabari goats. MC-Malabari control; MHS-Malabari heat stress and PGF2α-Prostaglandin F2α. Values bearing different superscripts differ significantly at $P < 0.05$.

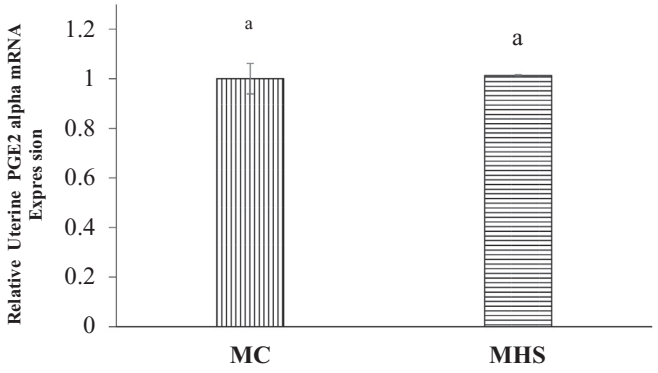

Fig. 1F. . Relative quantitative expression patterns of uterus PGE2 mRNA in both control and heat stressed Malabari goats.
Fig.1(F) depicts the impact of heat stress on the PGE2 mRNA expression patterns between control and heat stress group of Malabari goats. MC-Malabari control; MHS-Malabari heat stress and PGE2-Prostaglandin E2. The values bearing similar superscripts do not differ significantly.

patterns of COX-2 in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus COX-2 in control and heat stress groups are observed as 1.01 and 0.23 respectively. The heat stress significantly ($P < 0.05$) influenced the expression pattern of *COX-2* in Malabari goat with lower value established being in MHS group. Further, no significant correlation was established between THI and *COX-2* gene expression pattern (Table 3).

3.6. Relative prostaglandin F2α (PGF2α) mRNA expression pattern

Fig. 1(E) depicts the heat stress impact on the expression patterns of *PGF2α* in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus *PGF2α* in control and heat stress groups are 1.02 and 2.33 respectively. Further, it was evident that the *PGF2α* expression pattern was significantly ($P < 0.05$) up regulated in heat stress group as compared to the control group animals. Further, a strong negative correlation ($P < 0.01$) was established between THI and *PGF2α* gene expression pattern (Table 3).

3.7. Relative prostaglandin E2 (PGE2) mRNA expression pattern

Fig. 1(F) depicts the heat stress impact on the expression patterns of *PGE2* in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus *PGE2* in control and heat stress groups are 1.0 and 1.01 respectively. However, it was evident from the result that heat stress did not influence the expression pattern of *PGE2* gene in Malabari breed. This was evident from the comparable levels of *PGE2* expression pattern between the MC and MHS group. Further, a strong negative correlation ($P < 0.01$) was established between THI and *PGE2* gene expression pattern (Table 3).

4. Discussion

Most of the indigenous goat breeds are well known for their survival in their native tract and mostly the non-descript and other indigenous goat breeds are distributed in the tropical regions where generally adverse environmental condition persists which are not congenial for maintaining the productive performance. Malabari breed goat is one such breed in Southern India well

known for its adaptive capability in hot humid tropical environment [26]. Although there are several reports which established the adverse impacts of heat stress on the reproductive performance of goats [23–25], studies pertaining to heat stress influence on hypothalamic-pituitary-gonadal (HPG) axis associated genetic traits are very meagre. Therefore, the results obtained from the current study on the establishment of heat stress associated changes on the expression patterns of the various HPG axis genes is the first of its kind in domestic animals. This signifies the importance of this study to elucidate the molecular mechanisms associated with heat stress influenced reproductive performance in goats.

The THI followed in the study clearly established the heat stress for the animals as any cumulative value above 75 as per McDowell [19] model was considered extremely severe heat stress to animals and with the THI value of 86.5 recording during outside exposure in MHC group clearly indicated that these animals were subjected to extremely severe heat stress. This justifies the hypothesis of studying the reproduction related gene expressions in Malabari goats during heat stress exposure.

The physiological variables that could have been influenced by heat stress were not included in this study as it was already established in the same breed in our laboratory. In a series of studies conducted in Malabari breed, it was established that heat stress significantly influenced the body weight, body condition scoring (BCS), drinking frequency, water intake, respiration rate and rectal temperature [27,29]. Heat stress significantly reduced the body weight as well as BCS [27]. In an effort to establish the resilience capacity of Malabari breed Aleena et al. [28] reported that the heat stressed animals apart from exhibiting higher drinking frequency and water intake also showed significantly higher respiration rate and rectal temperature reflecting the adaptive potential of this breed to harsh climatic condition. Therefore, the current study was primarily targeted towards establishing the heat stress impact on reproduction related gene expression.

The expression pattern of *FSHR* mRNA was significantly lower in MHS groups. It is a general finding that FSH secretion is elevated under heat stress condition probably due to reduced inhibition of negative feedback from smaller follicles which ultimately affect the reproductive efficiency of dairy animals [18,29]. Further, the significant positive correlation between THI and *FSHR* in the current study proves this. This could be to meet the huge demand for FSH action during heat stress exposure as is the case with indigenous animals which tries to adapt and maintain production. The heat stress induced reduced FSHR expression could be attributed to the over utilization of these receptors by the increased FSH concentration. The heat stress induced reduced *FSHR* expression could culminate in the lack of sufficient FSH stimulation to increase the aromatase activities leading to reduced estradiol production reflecting the poor reproductive performance of the animals [13].

The *LHR* mRNA expression pattern also was similar to that of FSHR with significantly lower expression recorded in MHS group. This indicates that heat stress negatively influenced the expression pattern of *LHR* in Malabari goats. Similarly, there are reports which established lower *LHR* in heat stressed goats [13,30]. This was attributed to the altered follicular functions during heat stress, including follicular dynamics and steroidogenic activity and this has been described as a major factor in inducing reduced summer fertility [13,31,32]. The heat stress induced reduced *LHR* could be the reason for suppressed follicular responsiveness to LH. This culminates in lack of sufficient LH stimulation leading to regression of the follicles before ovulation thus resulting in ovulation failure ultimately resulting in reduced reproductive performance [33,34].

The ESTRα expression was higher in MHS group as compared to MC group. There are no reports available on the influence of heat stress on ESTRα expression in ruminant livestock. Therefore, the current study is the first of its kind to establish the higher expression pattern of ESTRα in MHS group. The ESTRα was found to be the predominant receptor subtype controlling the reproductive performance in animals [35]. The very high expression of this receptor in heat stressed goats signifies the importance of this receptor in controlling the reproductive activities of Malabari goats. The significantly higher expression pattern of ESTRα gene in MHS group goats could indicate the non-utilization of these receptors and this could be attributed to the significantly lower estradiol concentration in heat stressed animals [3,13,36].

The *COX-2* was considered developmentally important gene that transcribes an enzyme related to prostaglandin synthesis [37]. A similar mRNA expression pattern like that of FSHR and LHR were obtained for *COX-2* gene expression with significantly lower expression recorded in MHS group. Similarly, El-Sayed et al. [38] reported significantly lower *COX-2 gene* expression in Jersey cows while found no effect on the expression pattern of *COX-2* in heat stressed Nellore cow. This difference in expression pattern of *COX-2 gene* between Jersey and Nellore breeds of cattle could be attributed to the breed differences. Further, the non-significant influence of heat stress on *COX-2 gene* expression could be attributed to the resilience capacity of Malabari goat for its survival in tropical environment.

Prostaglandins (PGs) produced by endometrium serves as a crucial mediators in maternal recognition of pregnancy, implantation and parturition [39]. The *PGF2α* mRNA expression was significantly higher in MHS group as compared to MC group. Exposure of heat stress in the present study resulted in marked increase in the expression pattern of *PGF2α* from the endometrium. Similar finding of heat stress induced increase in production of *PGF2α* in endometrium was reported in dairy cow [40] and sheep [41]. However, Kobayashi et al. [42] reported contrasting result of no effect of temperature stress on *PGF2α* production. Increased *PGF2α* synthetic capacity of endometrium exposed to heat stress may be due to heat-induced alterations in endometrial cellular membranes resulting in increased mobilization of substrate for prostaglandin biosynthesis [41]. As maintenance of luteal function is associated with alterations in endometrial prostaglandin production and increased prostaglandin secretion following heat stress may compromise corpus luteum (CL) function and initiate luteal regression [43].

Heat stress may be a cause of summer infertility by decreasing the oviductal motility via promoting *PGE2* production [42]. Thermal exposure did not influence the expression pattern of *PGE2* in the endometrial cells between the groups. Similarly, Mondal et al. [41] did not observe any difference in the expression pattern of *PGE2* in sheep endometrium. However, Kobayashi et al. [42] also reported increased *PGE2* production in the heat stressed dairy cow. They attributed this heat stress induced increased *PGE2* production to either increase in the expressions of PGESs or increase in the PGES activity. Further, a strong negative correlation of THI with both *PGF2α* and *PGE2* also indicates the detrimental effect of high temperature and humidity to the reproductive efficiency of Malabari breed goat.

5. Conclusion

The study is the first of its kind to establish the impact of heat stress on the reproductive activity related candidate gene expressions in goats. The results from the study clearly indicated that heat stress brought in adverse impact on reproductive efficiency by altering the expression patterns of *FSHR, LHR, ESTRα, COX-2 and PGF2α*. This finding is further supported by the strong correlation of THI with *FSHR, ESTRα, PGF2α, and PGE2 genes*. However, THI did not show any correlation with LHR and COX-2. Therefore, this

preliminary study points towards the use of expression patterns *FSHR*, *ESTRα*, and *PGF2α* to be the indicators of heat stress effect on reproductive performance in indigenous goats as these variables showed significant influence for heat stress treatment as well as a strong correlation with THI.

Disclosure statement

The authors declare that there is no any conflict of interest for this manuscript.

Acknowledgements

The authors are more grateful to the Director, ICAR-National Institute of Animal Nutrition and Physiology for providing the research facilities to complete this study. The authors also highly thankful to the Indian Council of Agricultural Research (ICAR) for providing the funding for this research work.

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